COMMISSION OF THE EUROPEAN COMMUNITIES

COM(81) 128 final

Brussels, 24 March 1981

Proposal for a

COUNCIL DIRECTIVE

amending Directive 73/405/EEC on the approximation of the laws of the Member States relating to methods of tesring the biodegradability of anionic surfactants

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(submitted to the Council by the Commission)

COM(81) 128 final

Explanatory Memorandum

I. General

Council Directive 73/405/EEC relating to methods of testing the biodegradability of anionic surfactants [1], which is to be amended by this proposal, forms part of the general programme for the removal of technical barriers to trade approved by the Council on 28 May 1969. There are so far two Directives on detergents (specific washing and cleaning agents). The Commission transmitted a proposal for a third Directive to the Council for approval on 18 February 1980.
All these Directives have two main aims: to improve environmental protection and to secure free trade within the European Community.

The main purpose of the present proposal is to adapt to technical progress the method described in the Annex (confirmatory test procedure) for testing the biodegradability of anionic surfactants. The need for this adaptation became obvious during the preparation of the proposal on methods of testing non-ionic surfactants put before the Council a year ago. The confirmatory test procedures proposed in the two Directives must correspond as far as possible. This test is to be carried out in the event of objections pursuant to Article 5 (2) of Directive 73/404/EEC on detergents[3], and serves as a basis for the Commission's decision.

This proposal also updates the measuring methods used in the Member States and incorporates an additional method.

The opportunity is also taken to define the scope of the Directive more precisely as the existing text has given rise to difficulties of interpretation.

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1 OJ N° L 347, 17.12.1973, p. 53
2 OJ N° C 104, 28. 4.1980, p. 112
3 OJ N° L 347, 17.12.1973, p. 51

II. Comments on the Articles

Article 1

The words added to Article 1 are intended to define the scope of the Directive more precisely. They make it clear that the Directive applies only to surfactants used in detergents. Consequently the Directive does not apply to surfactants in products other than detergents, a point that was not clear in the existing text.

In addition, the references to existing methods in certain Member States are brought up to date and the method in use in the United Kingdom is incorporated. The text of the existing Articles 2 and 3 is also made more precise in the light of experience gained.

Article 2

The reference method described in the Annex (confirmatory test procedure) is adapted, on the lines of the method to be used for non-ionic surfactants, to take account of the latest developments in science and technology. In future, adaptations to technical progress will be made by the Committee procedure laid down in the proposal for a third Directive mentioned in Section I above.

Articles 3 and 4

These Articles are common to all Directives.

III. Consultation of the parties concerned

The proposal for a Directive was drafted in close cooperation with experts from the Member States. Account was taken of the opinions given by representatives of the relevant industries.

IV. Consultation of the European Parliament and of the Economic and Social Committee

Pursuant to the second paragraphe of Article 100 of the EEC Treaty, the opinions of these two bodies are required.

Proposal for a

COUNCIL DIRECTIVE

amending Directive 73/405/EEC on the approximation of the laws of the Member States relating to methods of testing the biodegradability of anionic surfactants

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 100 thereof,

Having regard to the proposal of the Commission,

Having regard to the Opinion of the European Parliament,

Having regard to the Opinion of the Economic and Social Committee,

Whereas Council Directive 73/405/EEC[1] must be adapted to take account of the latest developments in science and technology, thus necessitating:

- the updating of the references relating to the methods listed in Article 2;
- the addition to Article 2 of a further method of measurement which is in use in the United Kingdom;
- the improvement of the 'Confirmatory test procedure' provided for in the event of a dispute;

Whereas pursuant to Article 4 of Council Directive 73/404/EEC of 22 November 1973 on the approximation of the laws of the Member States relating to detergents[2] suitable tolerances for the measurement of biodegradability should be determined in order to take due account of the unreliability of text methods which could result in rejection decisions with important economic consequences; whereas a rejection decision may therefore only be taken where the results obtained with one of the test methods specified in Article 2 of Directive 73/405/EEC reveal a biodegradability of less than 80%;

"Whereas certain confusion having arisen as to the scope of Directive 73/405/EEC, it is necessary to make quite clear that the Directive applies only to surfactants used in detergents; it is also necessary to make quite clear that it is the level of biodegradability of the anionic surfactants contained in a detergent, and not the level of biodegradability of the detergent itself, which is dealt with in Article 2;"

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[1] OJ No L 347, 17.12.1973, p. 53

[2] OJ No L 347, 27.12.1973, p. 51

HAS ADOPTED THIS DIRECTIVE :

Article 1

Directive 73/405/EEC is hereby amended as follows :

1. The following is added to Article 1 :
" used in detergents".

2. Articles 2 and 3 are replaced by the following :

" Article 2

Pursuant to Article 4 of Directive 73/404/EEC relating to detergents, due account being taken of the unreliability of the test methods, Member States shall prohibit the placing on the market and use on their territory of a detergent if the biodegradability of the anionic surfactants contained therein is less than 80 % determined in accordance with one of the following methods :

- the OECD method, published in the OECD's technical report of 11 June 1976 on a "Proposed method for the Determination of the Biodegradability of Surfactants used in Synthetic Detergents";
- the method in use in France, approved by decree of 28 December 1978 published in the "Journal Officiel de la République Française" of 18 January 1979, pages 514/515, and by experimental standard T 73-260 of February 1971 published by the "Association Française de Normalisation" (AFNOR);
- the method in use in the Federal Republic of Germany, laid down under the "Verordnung über die Abbaubarkeit anionischer und nichtionischer grenzflächenaktiver Stoffe in Wasch- und Reinigungsmitteln" of 30 January 1977 and incorporated in the Order amending that Order of 18 June 1980, as published in the Bundesgesetzblatt 1980, Part I, page 706;
- the method in use in the United Kingdom, called the "Porous Pot Test" and described in Technical Report No 70 (1978) of the Water Research Centre.

Article 3

Under the procedure laid down in Article 5 (2) of Directive 73/404/EEC relating to detergents, the laboratory opinion on anionic surfactants shall be based on the Reference method (confirmatory test procedure) described in the Annex to this Directive."

3. The Annex is replaced by the Annex to this Directive.

Article 2

Member States shall bring into force the provisions necessary in order to comply with this Directive not later than 1 June 1982 and shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

ANNEX

DETERMINATION OF THE BIODEGRADABILITY OF ANIONIC SURFACE ACTIVE AGENTS

REFERENCE METHOD (CONFIRMATORY TEST PROCEDURE)

CHAPTER 1

1.1. Definition

Anionic surface active agents in the sense of this directive are those surface active agents, which, after passage through cationic and anionic ion exchangers are separated by fractional elution and determined as methylene blue active substance (MBAS) according to the analytical procedure described in Chapter 3.

1.2. Equipment needed for measurement

The method of measurement employs the small activated sludge plant shown in Figure 1, and in greater detail in Figure 2.

The equipment consists of a storage vessel A for synthetic sewage, dosing pump B, aeration vessel C, settling vessel D, air-lift pump E to recycle the activated sludge, and vessel F for collecting the treated effluent.

Vessels A and F must be of glass or suitable plastic and hold at least 24 litres. Pump B must provide a constant flow of synthetic sewage to the aeration vessel; this vessel, during normal operation, contains 3 litres of mixed liquor. A sintered aeration cube G is suspended in the vessel C at the apex of the cone. The quantity of air blown through the aerator should be monitored by means of a flowmeter H.

1.3. Synthetic sewage

A synthetic sewage is employed for the test. Dissolve in each litre of tap water:

160 mg peptone

110 mg meat extract

30 mg urea $(CO(NH_2)_2)$

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7 mg sodium chloride (NaCl)

4 mg calcium chloride, ($CaCl_2.2H_2O$)

2 mg magnesium sulphate ($MgSO_4.7H_2O$)

28 mg of monohydrogen dipotassium ortho phosphate (K_2HPO_4)

and 20 ± 2 mg MBAS

The MBAS is extracted from the product to be tested by the method given in Chapter 2. The synthetic sewage is freshly prepared daily.

1.4. Preparation of samples

1.4.1. Uncompounded surfactants may be examined in the original state. The MBAS content must be determined in order to prepare the synthetic sewage (1.3).

1.4.2. Formulated products are analysed for MBAS and soap content. They must be subjected to an alcoholic extraction and separation of MBAS (see Chapter 2).

The MBAS content of the extract must be known in order to prepare the synthetic sewage.

1.5 Operation of equipment

Initially fill aeration vessel C and settling vessel D with synthetic sewage. The height of the vessel D should be so fixed that the volume contained in the aeration vessel C is 3 litres. Inoculation is made by introducing 3 ml of a secondary effluent of good quality, freshly collected from a treatment plant dealing with a predominantly domestic sewage. The effluent must be kept under aerobic conditions in the period between sampling and application. Then set the aerator, air lift E and dosing device B in operation. The synthetic sewage must pass through aeration vessel C at the rate of one litre per hour; this gives a mean retention time of 3 hours.

The rate of aeration should be regulated so that the contents of vessel C are kept constantly in suspension while the dissolved oxygen content is at least 2 mg/l. Foaming must be prevented by appropriate means. Antifoaming agents which inhibit the activated sludge or contain MBAS must not be used. The air-lift pump E must be set so that the activated sludge from the settling vessel is continually and regularly recycled to aeration vessel C. Sludge which has accumulated around the top of the aeration vessel C, in the base of the settling vessel D, or in the circulation circuit must be returned to the circulation at least once each day by brushing or some other appropriate means. When sludge

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fails to settle, its density may be increased by addition of 2 ml portions of a 5 per cent solution of ferric chloride, repeated as necessary.

The effluent from separator D is accumulated in vessel F for 24 hours, following which a sample is taken after thorough mixing. Vessel F must then be carefully cleaned.

1.6. Checking measuring equipment

The MBAS content (in mg/l) of the synthetic sewage is determined immediately before use.

The MBAS content (in mg/l) of the effluent collected over 24 hours in vessel F should be determined analytically by the same method, immediately after collection; otherwise the samples must be preserved, preferably by freezing. The concentrations must be determined to the nearest 0.1 mg/l MBAS.

As a check on the efficiency of the process the chemical oxygen demand (COD) or the dissolved organic carbon (DOC) of the filtered effluent accumulated in vessel F, is measured at least twice weekly, as well as that of the filtered synthetic sewage in vessel A.

The reduction in COD or DOC should level off when a roughly regular daily MBAS biodegradation is obtained i.e. at the end of the running-in period shown in Figure 3.

The loss on ignition of the dried suspended solids in the activated sludge in the aeration tank should be determined twice a week (in g/l). If it is more than 2.5 g/l, the excess activated sludge must be discarded.

The test is performed at room temperature; this should be steady and should be kept between 291 and 298K (18 - 25°C).

1.7. Calculation of biodegradation

The percentage biodegradation of MBAS must be calculated every day on the basis of the MBAS content in mg/l of the synthetic sewage and the corresponding effluent accumulated in vessel F.

The biodegradation figures thus obtained should be presented graphically as in Figure 3.

The biodegradation of the MBAS should be calculated as the arithmetic mean of the figures obtained over the 21 days which follow the running-in period, during which biodegradation has been regular and the operation of the plant trouble-free. In any case the duration of the running-in period should not exceed six weeks.

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The daily biodegradation values are calculated to the nearest of 0.1 per cent but the final result is given to the nearest whole number.

In some cases it may be permissible to reduce the frequency of sampling but at least 14 results collected over the 21 days which follow the running-in period should be used in calculating the average.

CHAPTER 2

PRELIMINARY TREATMENT OF PRODUCTS TO BE TESTED

2.1. Preliminary notes

2.1.1. Treatment of samples

The treatment of anionic surface active agents and formulated detergents prior to the determination of biodegradability in the confirmatory test is:-

PRODUCTS	TREATMENT
Anionic surfactants	None
Formulated detergents	alcoholic extraction followed by ion exchange separation and fractional elution from the anion exchanger

The purpose of the alcoholic extraction is to eliminate the insoluble and inorganic ingredients of the commercial product which in some circumstances might upset the biodegradability test.

2.1.2. Ion exchange procedure

Isolation and separation of anionic surface active agents from soap, nonionics and cationics are required for correct biodegradability tests.

This is achieved by an ion exchange technique using a macroporous anion exchange resin and suitable elu-ants for fractional elution. Thus soap, anionic and nonionic surfactants may be isolated in one procedure.

2.1.3. Analytical control

After homogenising, the content of anionic surfactants in the synthetic detergent is determined according to the MBAS analytical procedure. The soap content is determined by a suitable analytical method. This analysis of the products is necessary to calculate the quantities required to prepare fractions for the biodegradability test.

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Quantitative extraction is not necessary; however at least 80% of the anionic surfactants should be extracted. Usually, 90% or more is obtained.

2.2. Principle

From an homogeneous sample (powders, dried pastes, and dried liquids) an ethanol extract is obtained which contains the surfactants, soap and other alcohol-soluble constituents of the synthetic detergent sample.

The ethanol extract is evaporated and dissolved in an isopropanol/water mixture, and the solution obtained is passed through a strongly acidic cation exchange/macro-porous anion exchange combination heated to 323 K (50°C). This temperature is necessary to prevent precipitation of fatty acids in acidic media.
The nonionic surfactants remain in the effluent.
The soap fatty acids are separated by elution with ethanol containing CO_2. The anionic surfactants are then obtained as ammonium salts by elution with an aqueous isopropanolic solution of ammonium bicarbonate. These ammonium salts are used for the degradation test.

Cationics, which might upset the biodegradability test and the analytical procedure, are eliminated by the cation exchanger placed on top of the anion exchanger.

2.3. Chemicals and equipment

2.3.1. Deionised water

2.3.2. Ethanol, 95% (v/v) C_2H_5OH
(permissible as denaturant: methyl ethyl ketone or methanol)

2.3.3. Isopropanol/water mixture (50/50):
50 parts by volume isopropanol ($CH_3CHOH.CH_3$) and
50 parts by volume water (2.3.1.).

2.3.4. Solution of carbon dioxide in ethanol (approx. 0.1% CO_2):
using a delivery tube with a built-in frit, pass carbon dioxide (CO_2) through the ethanol (2.3.2) for 10 minutes. Use fresh solutions only.

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2.3.5. Ammonium bicarbonate solution (60/40):
0.3 mol NH_4HCO_3 in 1000 ml isopropanol/water mixture of 60 parts by volume isopropanol and 40 parts by volume water (2.3.1.)

2.3.6. Cation exchanger (KAT), strongly acidic, resistant to alcohol (50 - 100 mesh).

2.3.7. Anion exchanger (AAT), macro-porous, Merck Lewatit MP 7080 (70 - 150 mesh) or equivalent.

2.3.8. Hydrochloric acid, 10% HCl (w/w)

2.3.9. 2000 ml round-bottomed flask with ground glass stopper and reflux condenser

2.3.10. 90 mm dia. suction filter (heatable) for paper filters

2.3.11. 2000 ml filter flask

2.3.12. Exchange columns with heating jacket and cock:
Inner tube 30 mm in diameter and 200 mm in height (Fig. 4).

2.3.13. Water-bath

2.3.14. Vacuum drying oven

2.3.15. Thermostat

2.3.16. Rotary evaporator

2.4. Extraction and Separation of Anionic surface active agents

2.4.1. Preparation of extract

The quantity of surface active agents necessary for the biodegradation test is about 50 g MBAS.

Normally the quantity of product to be extracted will not exceed 1000 g, but it may be necessary to extract further quantities of sample.
For practical reasons 5000 g will in most circumstances be the upper li

In preparing extracts for biodegradability test, experience has shown that there are advantages in using a number of small extractions rather than one large extraction.

The exchanger quantities specified are designed for a working capacity of 600-700 mmoles of surfactants and soap.

2.4.2. Isolation of alcohol-soluble constituents

Extract 250 g aliquots of sample by adding to 1250 ml portions of ethanol, heat the mixture to boiling point and reflux for 1 hour with stirring.

Pass the hot alcoholic solution through a coarse-pored suction filter heated to 323 K (50°C) and filter rapidly. Wash the flask and suction filter with approx. 200 ml hot ethanol. Collect the filtrate and filter washings in a filter flask.

In the case of pastes or liquid products, make sure that not more than 55 g anionic surfactant and 35 g soap are contained in the sample. Evaporate this weighed sample to dryness. Dissolve the residue in 2000 ml ethanol and proceed as described above.

Evaporate the ethanolic filtrate to dryness, preferably by means of a rotary evaporator. Repeat the operation if a greater quantity of extract is required. Dissolve the residue in 5000 ml isopropanol/water mixture.

2.4.3. Preparation of Ion exchange columns

Cation exchange column

Place 600 ml cation exchange resin (2.3.6.) in a 3000 ml beaker and cover by adding 2000 ml hydrochloric acid (2.3.8.). Allow to stand for at least 2 hours stirring occasionally. Decant the acid and transfer the resin into the column (2.3.12) by means of deionised water. The column should contain a glass wool plug. Wash the column with deionised water at the rate of 10 - 30 ml/min until the eluate is free of chloride. Displace the water with 2000 ml isopropanol/water mixture (2.3.3.) at a rate of 10 - 30 ml/min. The exchange column is now ready for use.

Anion exchange column

Place 600 ml anion exchange resin (2.3.7.) in a beaker and cover by adding 2000 ml deionised water. Allow the exchanger to swell for at least 2 hours. Transfer the resin into the column by means of deionised water. The column should contain a glass wool plug as exchanger supporting layer.

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Wash the column with 0.3 M ammonium bicarbonate solution (2.3.5.) until free of chloride. This requires about 5000 ml solution. Wash again with 2000 ml deionised water. Displace the water with 2000 ml isopropanol/water mixture (2.3.3.) at the rate of 10-30 ml/min. The exchange column is now in the OH-form and ready for use.

2.4.4. Ion exchange procedure

Connect the exchange columns so that the cation exchange column is placed on top of the anion exchange column. Using a thermostat heat the exchange columns to 323 K (50°C). Heat 5000 ml of the solution obtained in 2.4.2. to 333 K (60°C) and pass the solution through the exchanger combination at the rate of 20 ml/min. Wash the columns with 1000 ml hot isopropanol/water mixture (2.3.3.).

To obtain the anionic synthetic surfactants (MBAS), disconnect the KAT column. Using 5000 ml ethanol/CO_2 solution (323 K; 50°C) (2.3.4.), elute the soap fatty acids out of the AAT column. Reject the eluate.

Then elute the MBAS out of the AAT column with 5000 ml ammonium bicarbonate solution (2.3.5.). Evaporate the eluate to dryness on a steam bath or in a rotary evaporator. The residue contains the MBAS (as ammonium salt) and possibly non-surfactant anionics which have no detrimental effect on the biodegradation test. Add deionised water to the residue until a definite volume is obtained and determine the MBAS content in an aliquot as in Chapter 3. The solution is used as a standard solution of the anionic synthetic detergents for the biodegradation test. The solution should be kept at a temperature below 278 K (5°C).

2.4.5. Regeneration of ion exchange resins

The cation exchanger is rejected after use.

The anion exchange resin is regenerated by passing about 5000-6000 ml of ammonium bicarbonate solution (2.3.5.) down the column at a flow rate of approximately 10 ml/min until the eluate is free from anionics (methylene blue-test). Then pass 2000 ml isopropanol/water mixture (2.3.3.) down the anion exchanger to wash. The anion exchanger is again ready for use.

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CHAPTER 3

DETERMINATION OF ANIONIC SURFACE ACTIVE AGENTS IN BIODEGRADABILITY TEST

3.1. Principle

The method is based on the fact that the cationic dye methylene blue forms blue salts with anionic surfactants which can be extracted with chloroform. To eliminate interferences, the extraction is first effected from alkaline solution and the extract is then shaken with acidic methylene blue solution. The absorbance of the separated organic phase is measured photometrically at the wavelength of maximum absorption of 650 nm.

3.2. Reagents and equipment

3.2.1. Buffer solution pH 10:
Dissolve 24 g sodium bicarbonate ($NaHCO_3$) A.R. and 27 g anhydrous sodium carbonate (Na_2CO_3) A.R. in deionised water and dilute to 1000 ml.

3.2.2. Neutral methylene blue solution:
Dissolve 0.35 g methylene blue (B.P. grade) in deionised water and dilute to 1000 ml. Prepare the solution at least 24 hours before use. The absorbence of the blank chloroform phase, measured against chloroform, must not exceed 0.015 per 1 cm of layer thickness at 650 nm.

3.2.3. Acidic methylene blue solution:
Dissolve 0.35 g methylene blue (B.P. grade) in 500 ml deionised water and mix with 6.5 ml H_2SO_4. (d = 1.84). Dilute with deionised water 1000 ml. Prepare the solution at least 24 hours before use. The absorbence of the blank chloroform phase, measured against chloroform, must not exceed 0.015 per 1 cm of layer thickness at 650 nm.

3.2.4. Trichloromethane (Chloroform) $CHCl_3$ or Dichloromethane (Methylene dichloride) CH_2Cl_2, freshly distilled.

3.2.5. Dodecyl benzene sulphonic acid methyl ester

3.2.6. Ethanolic potassium hydroxide solution, KOH 0.1 M

3.2.7. Ethanol pure, C_2H_5OH

3.2.8. Sulphuric acid, H_2SO_4 0.5 M

3.2.9. Phenolphthalein solution:
Dissolve 1 g phenolphthalein in 50 ml ethanol and add 50 ml deionised water while stirring continuously. Filter off any precipitate obtained.

3.2.10. Methanolic hydrochloric acid: 250 ml hydrochloric acid conc. A.R. and 750 ml. methanol.

3.2.11. Separating funnel, 250 ml

3.2.12. Volumetric flask, 50 ml

3.2.13. Volumetric flask, 500 ml

3.2.14. Volumetric flask, 1000 ml

3.2.15. Weighing pipette

3.2.16. Round-bottomed flask with ground glass stopper and reflux condenser, 250 ml; boiling granules

3.2.17. pH-meter

3.2.18. Photometer for measurements at 650 nm, with 1 to 5-cm cells

3.2.19. Qualitative filter paper

3.3. Procedure

The samples for analysis must not be taken through a layer of foam.

After thorough cleaning with water, the equipment used for the analysis must be thoroughly rinsed with methanolic hydrochloric acid 3.2.10 and then with deionised water before using.

Filter the activated sludge plant influent and effluent to be examine immediately on sampling. Discard the first 100 ml of the filtrates.

Place a measured volume of the sample, neutralised if necessary, into a 250 ml separating funnel (3.2.11.). The volume of sample should contain between 20 and 150 µg of MBAS. At the lower MBAS content, up to 100 ml of sample may be used. When using less than 100 ml, dilute to 100 ml with deionised water. Add to the sample 10 ml of buffer solution (3.2.1.), 5 ml of neutral methylene blue solution (3.2.2.) and 15 ml of chloroform (or dichloromethane) (3.2.4.). Shake the mixture uniformly and not too vigorously for 1 minute. After phase separation, run the chloroform layer into a second separating funnel containing 110 ml of deionised water and 5 ml of acidic methylene blue solution (3.2.3.). Shake the mixture for 1 min. Pass the chloroform layer through a cotton-wool filter wetted with chloroform into a graduated flask (3.2.12.).

Extract the alkaline and acid solutions three times, using 10 ml of chloroform for the second and third extractions. Filter the combined chloroform extracts through the same cotton wool filter and dilute to the mark in the 50 ml-flask (3.2.12) with chloroform used for rewashing the cotton wool. Measure the absorbance of the chloroform solution with a photometer at 650 nm in 1- to 5-cm cells against chloroform. Run a blank determination through the whole procedure.

3.4. Calibration curve

Prepare a calibration solution from the standard substance dodecyl benzene sulphonic acid methyl ester (tetrapropylene type Mol.Wt 340) after saponification into the potassium salt. The MBAS is calculated as sodium dodecyl benzene sulphonate (Mol.Wt 348).

From a weighing pipette, weigh 400 to 450 mg of dodecyl benzene sulphonic acid methyl ester (3.2.5.) to the nearest 0.1 mg in a round-bottomed flask and add 50 ml of ethanolic potassium hydroxide solution and some boiling granules. After mounting the reflux condenser, boil for 1 hour. After cooling, wash the condenser and ground glass joint with about 30 ml of ethanol, and add these washings to the contents of the flask. Titrate the solution with sulphuric acid against phenolphthalein until it becomes colourless. Transfer this solution to a 1000 ml graduated flask (3.2.14.), dilute to the mark with deionised water and mix.

Part of this surfactant stock solution is then further diluted. Withdraw 25 ml, transfer to a 500 ml graduated flask (3.2.13.), dilute to the mark with deionised water and mix.

This standard solution contains $\frac{E \times 1.023}{20000}$ mg MBAS per ml, where E is the sample weight in mg.

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To establish the calibration curve, withdraw 1, 2, 4, 6, 8 ml each of the standard solution and dilute each to 100 ml with deionised water. Then proceed as stated under item 3.3 including a blank determination.

3.5. Calculation of results

The amount of anionic surfactant in the sample as MBAS is read from the calibration curve (3.4.). The MBAS content of the sample is given by:

$$\frac{\text{mg MBAS} \cdot 1000}{V} = \text{mg MBAS/l}$$

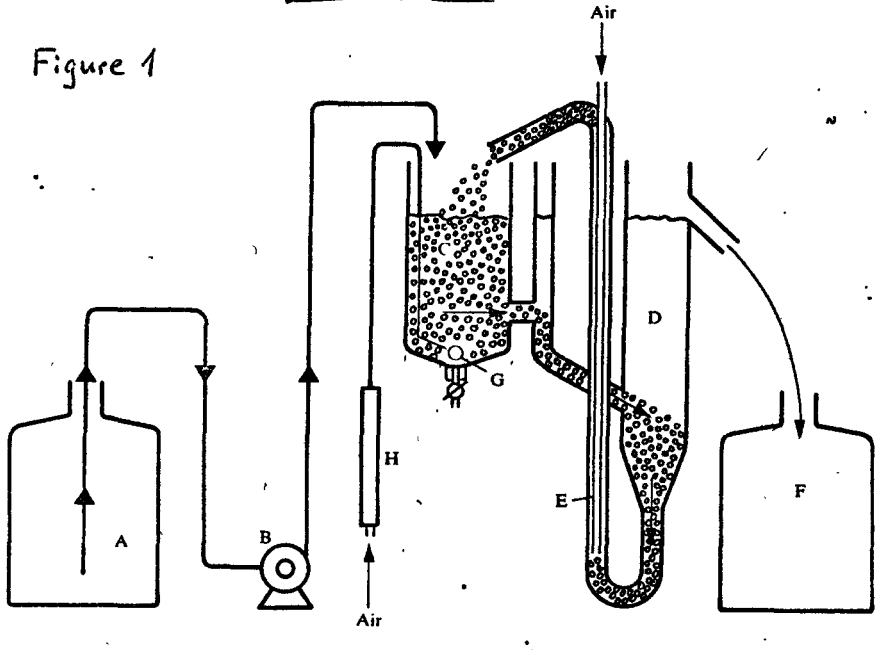
where V = ml volume of the sample used.

Express the results as sodium dodecyl benzene sulphonate (MW 348).

3.6. Expression of results

Express the results as mg MBAS/l to the nearest 0.1.

Figure 1



A. Storage vessel
B. Dosing device
C. Aeration chamber (three litres capacity)
D. Settling vessel
E. Air-lift pump
F. Collector
G. Aerator
H. Air-flow meter

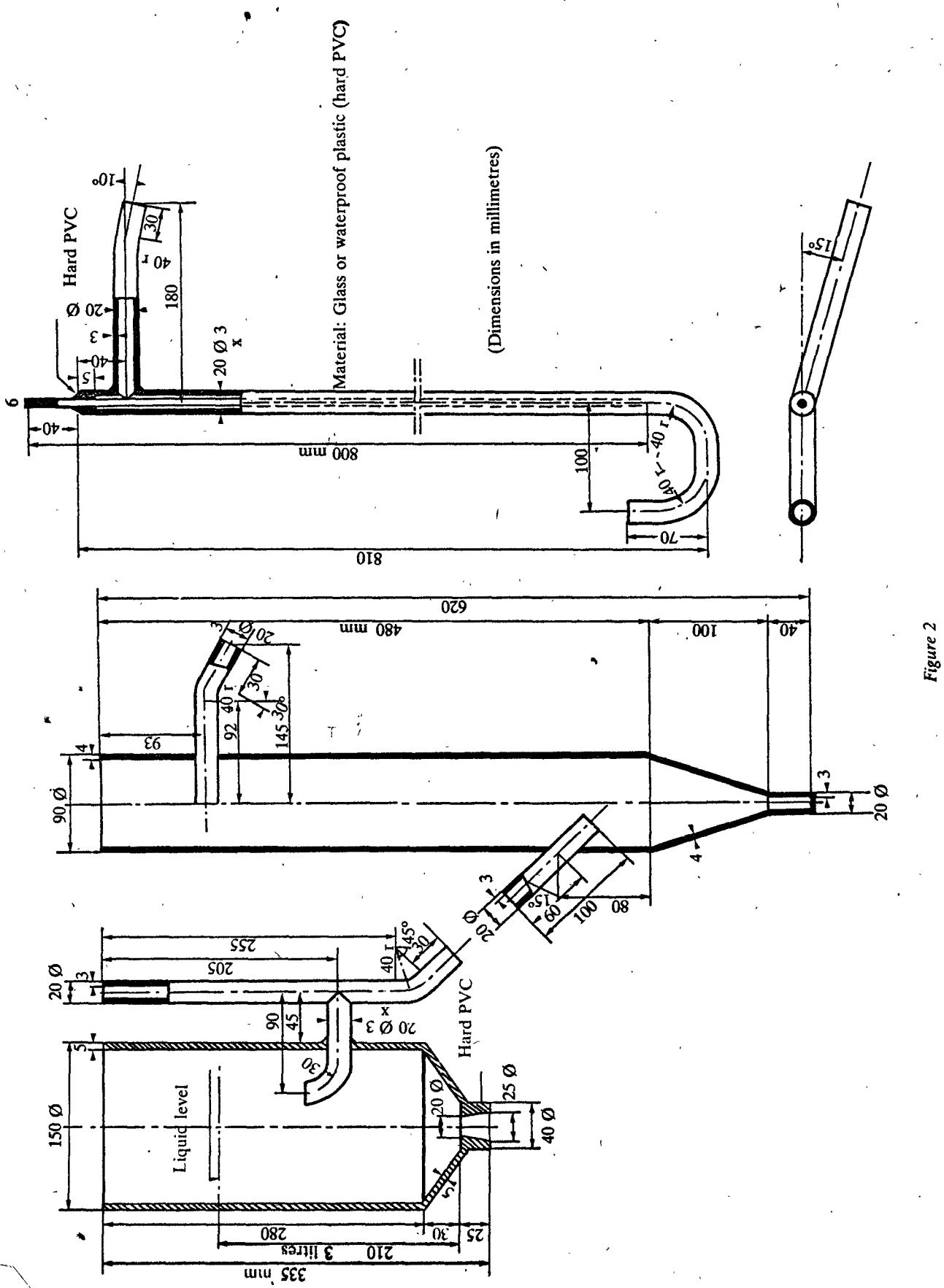

Material: Glass or waterproof plastic (hard PVC)
(Dimensions in millimetres)
Hard PVC
Liquid level
3 litres
335 mm
810
800 mm
480 mm
620

Figure 2

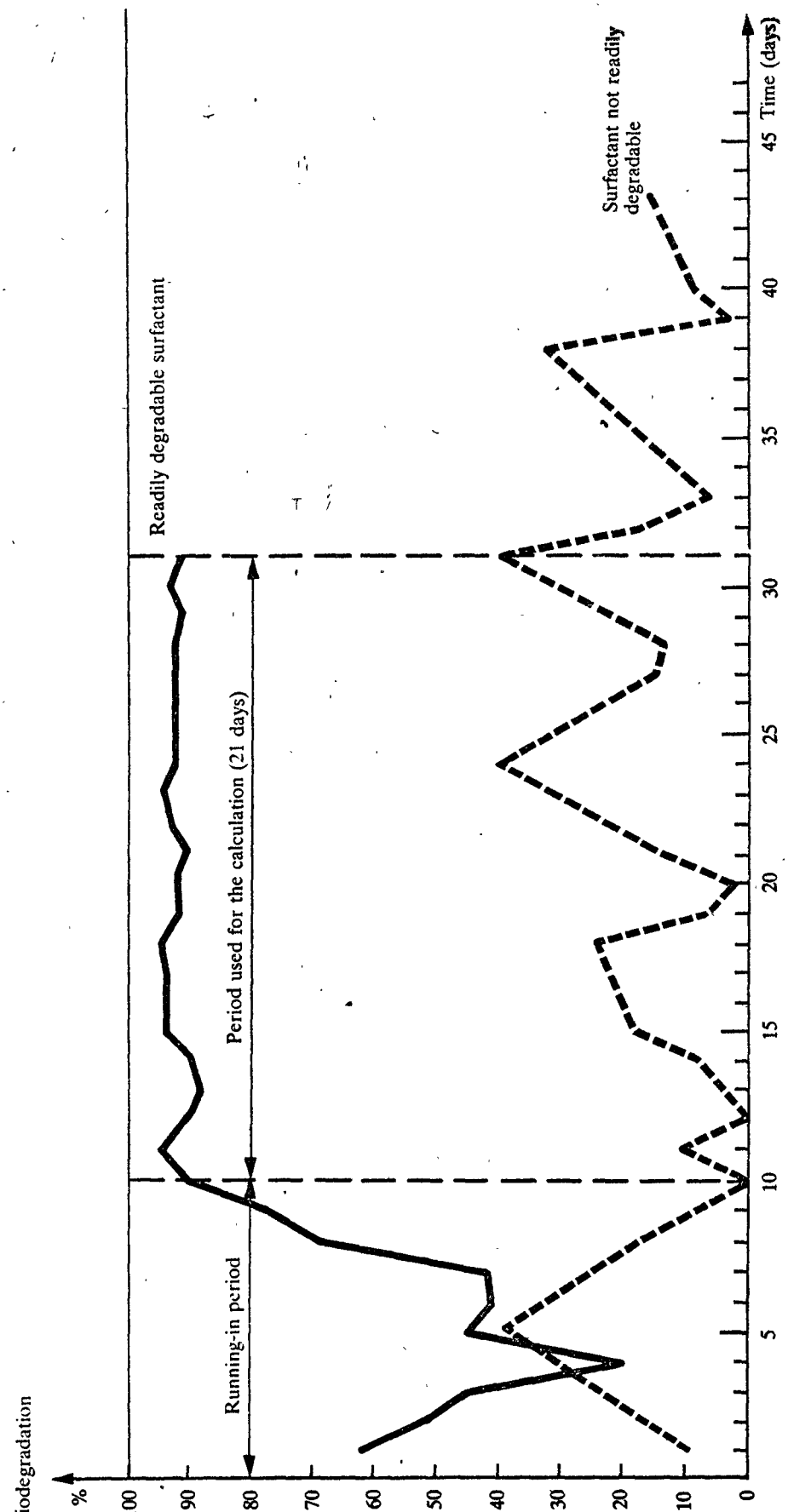


Figure 3

Calculation of biodegradability – Confirmatory test

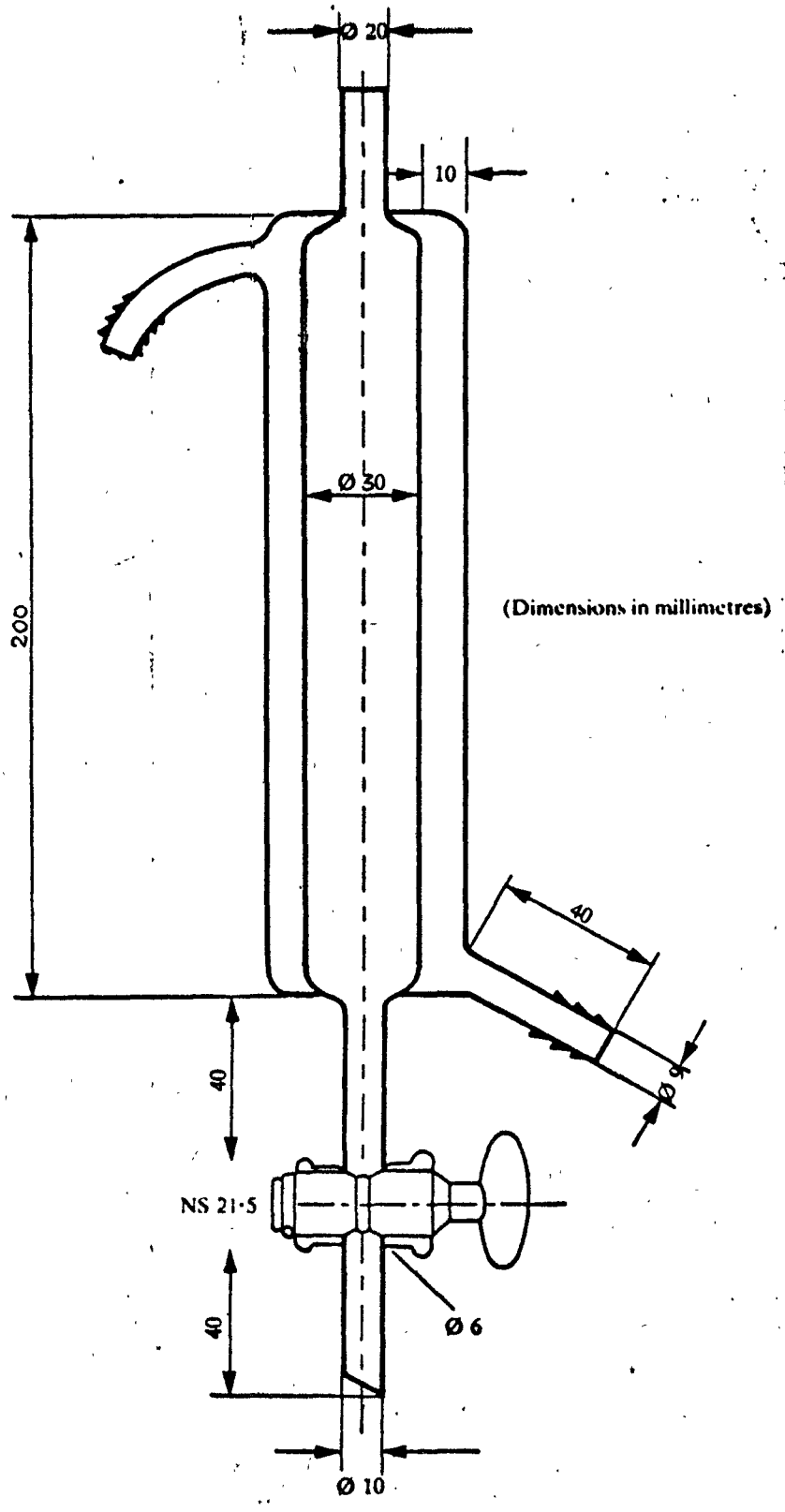


Figure 4

Heated exchange column